

[1-¹⁴C]-isopentenyl diphosphate (1 Ci/mol) 25 nmol
 Allylic diphosphate (geranyl diphosphate) 25 nmol
 Potassium phosphate buffer (pH 5.8) 10 mM
 MgCl₂ 5 mM
 Enzyme solution 100 µg
 H₂O to make 200 µl

After the reaction is over, 200 µl of saturated NaCl was added to the reaction solution and 1 ml of water-saturated butanol was added thereto, which was then agitated, centrifuged, and separated into two phases. To 800 µl of the butanol layer obtained was added 3 ml of a liquid scintillator and then the radioactivity was measured by the scintillation counter. The result is shown in FIG. 2.

The mutant prenyl diphosphate synthase has exhibited a thermo stability which is equal to that of the native geranylgeranyl diphosphate synthase, and is higher than that of the farnesyl diphosphate synthase derived from *Bacillus stearothermophilus*.

The solvent is evaporated from the remainder of the butanol layer by purging nitrogen gas thereinto while heating the layer in order to concentrate to a volume of about 0.5 ml. To the concentrate were added 2 ml of methanol and one 5 ml of potato acid phosphatase solution (2 mg/ml potato acid phosphatase, 0.5 M sodium acetate (pH 4.7)) to effect the dephosphorylation reaction at 37° C. Subsequently the dephosphorylated reaction product was extracted with 3 ml of n-pentane.

10 This was concentrated by evaporating the solvent by purging nitrogen gas thereinto, which was then analyzed by TLC (reverse phase TLC plate: LKC18 (Whatman), development solvent: acetone/water=9/1). The developed dephosphorylated reaction product was analyzed by the Bio Image Analyzer BAS2000 (Fuji Photo Film) to determine the location of radioactivity. The result when geranyl diphosphate was used as the allylic substrate is shown in FIG. 3.

15 The reaction product of the mutant prenyl diphosphate synthase was shown to be a farnesyl diphosphate.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(iii) NUMBER OF SEQUENCES: 14

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 330 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
 (A) ORGANISM: *Sulfolobus acidocaldarius*
 (B) STRAIN: ATCC 33909

(ix) FEATURE:
 (A) NAME/KEY: Asp-rich domain
 (B) LOCATION: 82-86

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Met Ser Tyr Phe Asp Asn Tyr Phe Asn Glu Ile Val Asn Ser Val Asn
 5 10 15

Asp Ile Ile Lys Ser Tyr Ile Ser Gly Asp Val Pro Lys Leu Tyr Glu
 20 25 30

Ala Ser Tyr His Leu Phe Thr Ser Gly Gly Lys Arg Leu Arg Pro Leu
 35 40 45

Ile Leu Thr Ile Ser Ser Asp Leu Phe Gly Gly Gln Arg Glu Arg Ala
 50 55 60

Tyr Tyr Ala Gly Ala Ala Ile Glu Val Leu His Thr Phe Thr Leu Val
 65 70 75 80

His Asp Asp Ile Met Asp Gln Asp Asn Ile Arg Arg Gly Leu Pro Thr
 85 90 95

Val His Val Lys Tyr Gly Leu Pro Leu Ala Ile Leu Ala Gly Asp Leu
 100 105 110

Leu His Ala Lys Ala Phe Gln Leu Leu Thr Gln Ala Leu Arg Gly Leu
 115 120 125

Pro Ser Glu Thr Ile Ile Lys Ala Phe Asp Ile Phe Thr Arg Ser Ile
 130 135 140

Ile Ile Ile Ser Glu Gly Gln Ala Val Asp Met Glu Phe Asp Arg

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145	150	155	160
Ile Asp Ile Lys Glu Gln Glu Tyr Leu Asp Met Ile Ser Arg Lys Thr			
165	170	175	
Ala Ala Leu Phe Ser Ala Ser Ser Ser Ile Gly Ala Leu Ile Ala Gly			
180	185	190	
Ala Asn Asp Asn Asp Val Arg Leu Met Ser Asp Phe Gly Thr Asn Leu			
195	200	205	
Gly Ile Ala Phe Gln Ile Val Asp Asp Ile Leu Gly Leu Thr Ala Asp			
210	215	220	
Glu Lys Glu Leu Gly Lys Pro Val Phe Ser Asp Ile Arg Glu Gly Lys			
225	230	235	240
Lys Thr Ile Leu Val Ile Lys Thr Leu Glu Leu Cys Lys Glu Asp Glu			
245	250	255	
Lys Lys Ile Val Leu Lys Ala Leu Gly Asn Lys Ser Ala Ser Lys Glu			
260	265	270	
Glu Leu Met Ser Ser Ala Asp Ile Ile Lys Lys Tyr Ser Leu Asp Tyr			
275	280	285	
Ala Tyr Asn Leu Ala Glu Lys Tyr Tyr Lys Asn Ala Ile Asp Ser Leu			
290	295	300	
Asn Gln Val Ser Ser Lys Ser Asp Ile Pro Gly Lys Ala Leu Lys Tyr			
305	310	315	320
Leu Ala Glu Phe Thr Ile Arg Arg Arg Lys			
325	330		

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 993 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Sulfolobus acidocaldarius*
- (B) STRAIN: ATCC 33909

(ix) FEATURE:

- (A) NAME/KEY: Asp-rich domain coding
- (B) LOCATION: 246-258

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

ATGAGTTACT TTGACAACTA TTTAATGAG ATTGTTAATT CTGTAAACGA CATTATTAAG	60
AGCTATATAT CTGGAGATGT TCCTAAACTA TATGAAGCCT CATATCATTT GTTTACATCT	120
GGAGGTAAGA GGTTAACGACC ATTAATCTTA ACTATATCAT CAGATTATTATT CGGAGGACAG	180
AGAGAAAAGAG CTTATTATGAG AGGTGCAGCT ATTGAAGTTTC TTCATACCTT TACGCTTGTG	240
CATGATGATA TTATGGATCA AGATAATATC AGAAGAGGGT TACCCACAGT CCACGTGAAA	300
TACGGCTTAC CCTTAGCAAT ATTAGCTGGG GATTTACTAC ATGCAAAGGC TTTTCAGCTC	360
TTAACCCAGG CTCTTAGAGG TTTGCCAAGT GAAACCATAA TTAAGGCTTT CGATATTTTC	420
ACTCGTTCAA TAATAATTAT ATCCGAAGGA CAGGCAGTAG ATATGGAATT TGAGGACAGA	480
ATTGATATAA AGGAGCAGGA ATACCTTGAC ATGATCTCAC GTAAGACAGC TGCATTATTC	540
TCGGCATCCT CAAGTATAGG CGCACCTTATT GCTGGTGCTA ATGATAATGA TGTAAGACTG	600
ATGTCTGATT TCGGTACGAA TCTAGGTATT GCATTTACAGA TTGTTGACGA TATCTTAGGT	660
CTAACAGCAG ACGAAAAGGA ACTTGGAAAG CCTGTTTTA GTGATATTAG GGAGGGTAAA	720

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AAGACTATAC TTGTAATAAA AACACTGGAG CTTTGTAAG AGGACGAGAA GAAGATTGTC	780
CTAAAGGCGT TAGGTAATAA GTCAGCCTCA AAAGAAGAAT TAATGAGCTC AGCAGATATA	840
ATTAAGAAAT ACTCTTTAGA TTATGCATAC AATTTAGCAG AGAAATATTAA TAAAAATGCT	900
ATAGACTCTT TAAATCAAGT CTCCCTCTAAG AGTGATATAC CTGGAAAGGC TTTAAAATAT	960
CTAGCTGAAT TTACGATAAG AAGGAGAAAAA TAA	993

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 37
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CATACTTTTT TCCCTGTGGC TGATGATATC ATGGATC	37
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(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 37
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CATACTTTTT TCCCTGTGCT TGATGATATC ATGGATC	37
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(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 37
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CATACTTATT TCCTTGTGCT TGATGATATC ATGGATC	37
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(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 37
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CATACTTATT TCCTTGTGGC TGATGATATC ATGGATC	37
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(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 36
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GTCTTCATA CTTATTGCT TATTCATGAT AGTATT

36

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 33
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

ATTCATGATG ATCTTCCATC GATGGATCAA GAT

33

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TTTTTCCTTG TGGCTGATGA TATCATG

27

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TTTTTCCTTG TGCTTGATGA TATCATG

27

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

TATTTTCCTTG TGCTTGATGA TATCATG

27

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

TATTTTCCTTG TGGCTGATGA TATCATG

27